

SUBSTRATE COLOR MATCHING IN THE GRASSHOPPER, *CIRCOTETTIX RABULA* (ORTHOPTERA: ACRIDIDAE)

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ABSTRACT.— Mechanisms important in maintaining substrate color matching in the grasshopper, *Circotettix rabula*, were studied near Aspen, Colorado, during the summers of 1968-70. Studies concentrated on populations on gray shale and red sandstone substrates. In both areas, collections revealed appreciable numbers of mismatched phenotypes among all age groups. The possibility of developmental homochromy was examined by observation of nymphs held in rearing boxes on matching and contrasting soil colors. The behavioral selection of matching substrate colors was tested by preference experiments. While not negating the possibility of these mechanisms, results suggested that they were of minor importance. Predation experiments, using *Sceloporus* lizards, demonstrated significant levels of selective predation on mismatched nymphs on both red and gray substrates. Experiments with bird and mammal predators, using adult grasshoppers, gave similar results. Release-recapture experiments with marked adults in areas of red and gray substrates showed markedly higher disappearance rates for mismatched animals. These results are interpreted to indicate that selective predation on mismatched animals is a major factor in maintaining substrate color matching in this species.

Over the past 25 years, students of ecological genetics have documented several major cases of rapid evolutionary response by animal populations subjected to strong selective pressures. The most thoroughly investigated of these concern industrial melanism in moths (Kettlewell, 1961), pesticide resistance in a wide variety of animals (Crow, 1966), and the coloration and banding patterns of various land snails (Cain and Sheppard, 1954). For the Peppered Moth, *Biston betularia*, an industrial melanic, and for the land snail, *Cepaea nemoralis*, selective predation by vertebrates has been demonstrated (by experimentation and direct observation) to be an important factor in the observed evolutionary response (Kettlewell, 1955, 1956; Sheppard, 1951; Cain and Sheppard, 1954; Cain and Currey, 1968).

The phenomenon of substrate color matching has been recorded for many species of animals of open habitats and is especially common in Acridid grasshoppers (Rowell, 1971). For these animals, the mechanisms involved appear to be complex and varied, including—at least for different species—predator selection for crypsis, behavioral preference for color-matching substrates, and homochromic responses during individual development (*ibid.*). Acridid species showing spatial or temporal variation in substrate color matching may therefore be of major value in the study of evolutionary processes operating on organisms capable of behavioral or developmental modification of genetically based characteristics.

In the Roaring Fork Valley near Aspen, Colorado, populations of many animals of open habitats show differences in general body coloration correlated with soil and rock substrate color. The sub-

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strates involved vary strikingly in color as a result of the variety of geological formations exposed in the valley. The most extensive exposures near Aspen consist of red sandstones of the Maroon Formation, dark gray shales of the Mancos Formation, and Precambrian granites on which yellow to brown clay soils are developed. Smaller areas of dark basaltic lavas and white shales also occur in the lower part of the valley. Although sharp changes in substrate color sometimes exist at contacts between different parent materials, the presence of a variety of other bedrock formations and the mixing of various parental materials in glacial and alluvial deposits produce an additional variety of substrates of intermediate colors in different parts of the valley.

Correlation of general body color with substrate color has been observed in the lizards *Sceloporus undulatus* and *S. graciosus* (Coleman, 1968), and, by the authors, in various species of Acridid grasshoppers, including *Arphia conspersa* Scudder, *Circotettix rabula* Rehn and Hebert, *Cratypedes neglectus* (Thomas), and *Trimerotropis suffusa* (Scudder).

During the summers of 1968-70, the authors conducted studies of the role of various factors in the maintenance of substrate color matching in populations of the grasshopper *Circotettix rabula*, an Acridid of the subfamily Oedipodinae, in the vicinity of Aspen. These studies concentrated on populations on exposures of Mancos Shale (gray) and Maroon Sandstone (red). The Mancos Shale population was studied at a location 6 miles west of Aspen on Colorado State Highway 82 at Brush Creek. The Maroon Sandstone population was studied at an area of cliffs on the north side of the Roaring Fork River opposite the community of Snowmass, about 15 miles west of Aspen. Both study areas consisted of relatively open, dry habitats on hilly terrain, with the dominant vegetation being a complex mixture of sagebrush, oak-serviceberry, pinyon-juniper, and riparian elements.

PROCEDURE

At the outset, three major mechanisms that, separately or in combination, might account for the pattern of substrate color matching were recognized:

1. Homochromy (the adoption, through developmental process in the nymphs, of a body color matching that of the substrate upon which they are living). This mechanism has been demonstrated in many Acridid grasshoppers (Rowell, 1971).
2. Behavioral preference by individuals for substrates of matching color. Such preferences have been recorded for some Oedipodine grasshoppers (Rowell, 1971). This preference could presumably act in conjunction with either 1 (above) or 3 (below) to enhance the degree of color matching achieved by these mechanisms.
3. Predator selection favoring individuals closely matching the substrate in body color. This mechanism might operate in conjunction with 1 or 2 (above) by selection against individuals in which the mechanisms of homochromy or behavioral preference are imperfectly developed. Alternatively, selection may relate to matched and mismatched color pheno-

types resulting directly from genotypic differences in processes of pigment production.

The present studies were designed to investigate the relative importance of these possible mechanisms.

The hypothesis of developmental adoption of environmental color was tested by a combination of field and experimental studies. Nymphs were collected in both study areas during the period 22-25 June 1968. Total body length and color phenotype were recorded for each of these animals. These animals were visually assigned to four color phenotype classes: gray, yellow brown, brown, and red. These collections were made to determine the presence and general frequency of mismatched phenotypes in the two areas.

In addition, during the June and July of 1968, live nymphs from each study area were divided into two groups of equal size, and placed in separate rearing boxes with red and gray soils and correspondingly painted walls. Totals of 54 gray and 52 red nymphs were used in this experiment. Food and water were provided in each box. Although it proved impossible to rear many of these nymphs to adulthood, many completed one or more molts and were maintained for periods of up to four weeks. This experiment was performed to determine if changes in body color could be induced in nymphs of the size classes for which data on frequency of various color phenotypes had been obtained in the field observations.

To investigate hypothesis 2, that of possible behavior preferences for matching substrates, four substrate-color preference tests were conducted during the summer of 1968. Preferences were tested separately for nymphs and adults from the two study areas. Tests were conducted outdoors in 4'x8' boxes covered with gray fiberglass screening, in which equal areas of red and gray soils from the study areas were presented. These tests consisted of a series of 1-hour runs, with the position of the boxes being reversed between successive runs. Experimental animals were introduced at the boundary of the two substrate types at the start of each run, and their positions were noted at the end of the one-hour period.

Several laboratory and field experiments were conducted to evaluate the intensity of predation on animals of matched and mismatched body color. The most extensive series was conducted using the lizards *Sceloporus undulatus* and *S. graciosus* as predators on nymphs. These experiments were carried out in the covered 4'x8' boxes described above. A layer of red or gray soil was placed in the bottom of the box, and several piles of flat stones of matching color constructed near the center of the box to allow shelter for both lizards and grasshopper nymphs. This was necessary to minimize the tendency for animals to climb onto the walls and covers of the boxes (which were of different color). Equal numbers of red and gray nymphs were then placed in the box and lizards introduced so that a ratio of 6-8 nymphs per lizard was obtained. These experiments were terminated when approximately one half of the nymphs had been eaten. During the summer of 1968 experiments were conducted on gray substrates. Experiments on red substrates were performed

during all three summers to obtain an adequate number of observations. Experiments were carried out in a shaded, outdoor area during 1968, with the duration of each run being 24 hours. In 1969 and 1970, experiments were done indoors under artificial lights. The duration of runs in these experiments varied from 6-12 hours.

During the summer of 1969 two series of experiments were conducted using the least chipmunk, *Eutamias minimus*, and the American robin, *Turdus migratorius*, as predators. For these experiments, adult grasshoppers were used. Experiments using the least chipmunk (Hobbs, 1969) were conducted in the 4'x8' box described above. The flight wings and hind legs of the grasshoppers used in these tests were removed to minimize the effects of flight and rapid movement in attracting attention of the predators. Two grasshoppers, one red and one gray, were placed in the box on a given substrate color, and the predator introduced. The grasshopper first captured was then noted. A total of 25 such tests were performed on each substrate color. A similar procedure was followed in experiments using the American robin (Coate, 1969), except that tests were performed in a cage 6'x6'x6' in size.

Two field experiments were also conducted to compare the rates of disappearance of red and gray adults following their release in an area of one substrate color. The animals used in these experiments were captured in the two study areas at locations about one-half mile from the locations at which releases were subsequently made. Animals were marked with a spot of colored airplane dope on the ventral side of the thorax and were released on the same date on which they were captured.

During the summer of 1968 two releases were made on a Mancos Shale (gray) area. On 24-26 July, 37 males and 34 females from the Mancos Shale population and 54 males and 50 females from the Maroon Sandstone population were released. The second release, made on 6 August, consisted of 14 males and 13 females from the Mancos Shale population and 15 males and 30 females from the Maroon Sandstone population. Recapture samples were taken on 1-3 August, 15-16 August, and 21 August.

During the summer of 1969, releases were made on an area of Maroon Sandstone (red) substrate. On 16-17 July, 65 males and 53 females from the red sandstone population and 62 males and 55 females from the gray shale population were released. Recapture samples were obtained on 24 July, 29-31 July, and 7-8 August.

RESULTS

Totals of 48 nymphs from the Mancos Shale population and 70 nymphs from the Maroon Sandstone population were obtained in the field collections made in June 1968 (Table 1). The distribution of these animals among the four color phenotype classes was summarized separately for animals less than 12 mm and for those 12 mm or over in total length. These data indicate that an appreciable frequency of mismatched color phenotypes exists, especially among

TABLE 1. Frequencies of nymphs of different color in collections from Mancos Shale (gray) and Maroon Sandstone (red) areas, 22-25 June 1968.

Substrate	Size Class	Color Phenotype Class (Visual Groupings)				Total
		Gray	Yellow Brown	Light-Dark Brown	Red	
Mancos Shale	<12 mm	18	1	2	5	26
	>12 mm	21	0	0	1	22
	Total	39	1	2	6	48
Maroon Sandstone	<12 mm	0	0	16	12	28
	>12 mm	0	2	9	31	42
	Total	0	2	25	43	70

the smaller nymphs, in both populations. In the Mancos Shale area, for example, 5 of the 25 nymphs less than 12 mm in total length were red. When analyzed by a 2x2 contingency test, the frequencies of matched and totally mismatched phenotypes in the two size groups were found to be significantly different, or nearly so. For the Mancos Shale population the calculated chi square value of 3.80 was nearly significant at the 5 percent level; for the Maroon Sandstone population, the value of 5.55 was significant at the 5 percent level (5 percent critical chi square, with 1 d.f., equals 3.841). In both cases, the frequency of mismatched individuals was lower among the larger nymphs.

The nymphs maintained in rearing boxes on substrates of contrasting color, however, showed no visually detectable changes in body color, as compared with those kept on matching substrates.

In the four experiments designed to test for substrate-color preference by nymphs and adults, the total numbers of preference responses obtained per experiment varied from 58 to 96. However, chi square goodness-of-fit tests, comparing observed results and an expectation of equal numbers selecting each substrate, showed no significant preferences for substrate color by nymphs or adults from either population.

Results of the predation experiments with the lizards *Sceloporus undulatus* and *S. graciosus* demonstrated selective predation on nymphs of mismatched color (Table 2). On the gray substrate a 2x2 contingency test of the experimental results showed that the numbers of nymphs eaten and not eaten were significantly influenced by body color (1 percent level); a similar test for the red substrate showed significance at the 5 percent level.

Fewer data were obtained for the experiments using the American robin and least chipmunk as predators (Table 3). Except for the results of the tests with the least chipmunk on the gray substrate (significant at the 5 percent level) these results do not show significantly different preferences for matched and mismatched grasshoppers. However, the pattern shown by these data is similar to that obtained for the lizard predation experiments. A common feature of experiments with all of the predators used was a higher intensity of

TABLE 2. Results of experiments involving predation by *Sceloporus undulatus* and *S. graciosus* on nymphs of *Circotettix rabula* from Mancos Shale (gray) and Maroon Sandstone (red) populations.

	Gray substrate		Total	Red substrate		Total
	Eaten	Not eaten		Eaten	Not eaten	
Red nymphs	41	9	50	46	48	94
Gray nymphs	20	30	50	61	32	93
Total	61	39	100	107	80	187
Chi square	16.81 (1 d.f.)			4.65 (1 d.f.)		

TABLE 3. Results of experiments involving predation by the American robin and the least chipmunk on adults of *Circotettix rabula* from Mancos Shale (gray) and Maroon Sandstone (red) populations.

	Gray substrate		Total	Red substrate		Total
	Eaten	Not eaten		Eaten	Not eaten	
American robin						
Red adults	17	8	25	12	13	25
Gray adults	8	17	25	13	12	25
Total	25	25	50	25	25	50
Chi square	3.17 (1 d.f.)			0.05 (1 d.f.)		
Least chipmunk						
Red adults	18	7	25	9	16	25
Gray adults	7	18	25	16	9	25
Total	25	25	50	25	25	50
Chi square	4.00 (1 d.f.)			1.44 (1 d.f.)		

predation on mismatched animals on the gray substrate than on the red.

Data from the release and recapture experiments with marked adults on both substrates showed rapid decline in the ratio of mismatched to matched animals with time (Figs. 1, 2). To evaluate the significance of this change, Chi Square tests were performed on the observed frequencies of red and gray animals in the first recapture sample obtained after each release, using the release ratio of red and gray animals to provide the expected frequencies. For the 24-26 July release and the 1-3 August recapture samples on the Mancos Shale area (1968), the calculated Chi Square value was 8.74. For the 6 August release and the 15-16 August recapture sample, the calculated Chi Square value was 8.78. Both of these tests indicate a highly significant (1 percent level) change in the ratio of red to gray animals. For the 16-17 July release and the 24 July recapture sample on the Maroon Sandstone area (1969), the calculated Chi Square value was 4.66 (significant at the 5 percent level).

The ratio of mismatched to matched animals (Figs. 1, 2) declined exponentially, according to the formula

$$R_t = R_0 e^{bt}$$

Where: R_0 = Ratio on date of release

R_t = Ratio t days after release

b = Rate of change in mismatched/matched ratio per day.

A rough estimate of the average rate of change, b , in this ratio was

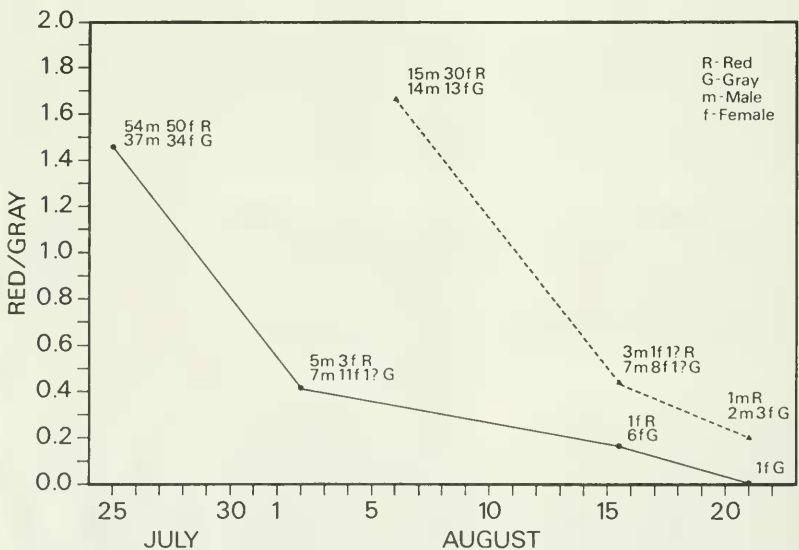


Fig. 1. Ratios of red (Maroon Sandstone) to gray (Mancos Shale) adults in the release-recapture experiment on the Mancos Shale study area.

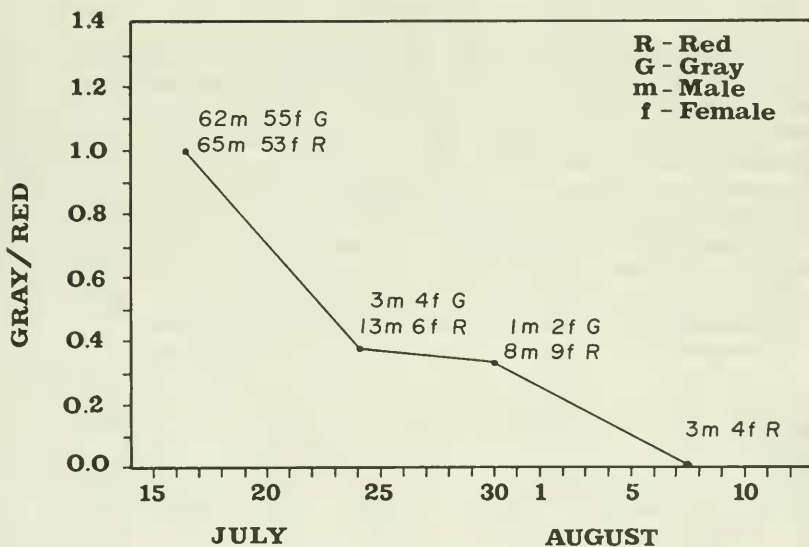


Fig. 2. Ratios of gray (Mancos Shale) to red (Maroon Sandstone) adults in the release-recapture experiment on the Maroon Sandstone study area.

obtained by calculating the least square linear regression of $\ln R$ on t . The slope of this relationship is a measure of b . Values for the rate of change in the red-gray ratio during the experiments initiated in July and August of 1968 on the Mancos Shale area were -0.10 and -0.14 , respectively. These values indicate that the disappearance rate of the red animals was of the order of 10 to 14 individuals per 100 individuals per day greater than that of the gray animals. On the Maroon Sandstone area, the differential rate of disappearance of the gray animals, calculated in a similar manner, was -0.09 , or 9 individuals per day greater than that of the red animals.

Recapture data from the field experiments were also analyzed with respect to frequencies of recapture of individuals of different sex. For the experiment on the Maroon Sandstone area, and for red animals in the Mancos Shale experiment no significant differences were noted between the sex ratios of animals released and those recaptured. However, for gray animals used in the Mancos Shale experiment, the sex ratio of the combined groups of released animals (24-26 July and 6 August 1968) was significantly different (Chi Square = 4.26, 5 percent critical value = 3.841) from that of the combined recapture samples for these releases. Males were less frequently recaptured than expected in this case. The males of this species perform a conspicuous aerial display, consisting of a prolonged dancing flight accompanied by a loud crackling call. This display is attractive to predators, and several observations of actual or attempted predation involved attempts by birds to capture such animals in flight.

DISCUSSION

Although the phenomenon of substrate color matching is well developed in *Circotettix rabula*, variation in body coloration exists in both nymphal (Table 1) and adult (Trent, 1968) populations in the areas examined in this study. Both of the study areas were adjacent to alluvial terraces of the Roaring Fork River and, thus, to substrates of somewhat different color. Furthermore, the adults are strong fliers, and in such situations it is likely that considerable dispersal occurs between areas differing in substrate color. During the summers of 1969 and 1970, direct observations of such dispersal were made near Aspen at a locality in which a whitish gray shale formation sharply contacts an exposure of Maroon Sandstone.

The specific locations from which collections of nymphs (Table 1) were made in 1968, however, were at least 100 m from areas of strongly differing substrate color. The occurrence of strongly mismatched forms among these nymphs, together with the failure of nymphs of comparable size to show modification of body color in the red and gray rearing boxes, suggests that any homochromic developmental response is relatively weak and that body color is to a large extent under direct genetic control. The possibility that artificial light conditions may have been unfavorable for homochromic response cannot be entirely dismissed, however. Because of the low mobility of the nymphs, it is probable that the existence of mismatched nymphs in the study areas is the result of dispersal of adults into the areas from other substrate types.

No evidence of behavioral selection of matching substrates was obtained in the laboratory preference tests. This evidence is further supported by the direct observations, cited above, of dispersal of adults between substrates of sharply differing color. Nevertheless, it is still possible that behavioral selection of matching substrates does occur to some degree, but was inhibited, especially for adults, by the artificial conditions of the test boxes.

Evidence of selective predation is strong. Field observations indicate that a variety of vertebrates prey on this species. Observations of either attempted or successful predation in the field were obtained for the sagebrush lizard, *Sceloporus graciosus*, the least chipmunk, *Eutamias minimus*, the western tanager, *Piranga ludoviciana*, the Steller's jay, *Cyanocitta stelleri*, the mountain bluebird, *Sialia cucurroides*, and the red-winged blackbird, *Agelaius phoeniceus*.

Results of the predator selection experiments (Tables 2, 3) indicate that differential predation is of major importance in the maintenance of substrate color matching. These observations correlate with the significantly lower frequency of mismatched nymphs in the larger size classes in the field (Table 1) and with the significantly greater rate of disappearance of mismatched adults in the release-recapture experiments.

In the release-recapture experiments, as conducted, it is impossible to rule out entirely the possibility of differential dispersal away from the release point by matched and mismatched animals. But,

the absence, or at least the weakness, of behavioral preferences for matching substrate color suggests that this is unlikely. The recapture data for males and females are also most consistent with the conclusion that predation is the primary factor in the differential disappearance rates of matched and mismatched adults. Males, which perform frequent and extensive aerial displays, should be both the most vulnerable to aerial predators and the most prone to dispersal from the release site. Mismatched males should then have the greatest opportunity for encountering and selecting more appropriate substrates. However, the only instance in which the disappearance rate for males significantly exceeded that for females was for gray adults on the gray substrate. If dispersal, combined with behavioral selection of matching substrates, had been of major importance, differences in male and female disappearance rates should have been greatest for mismatched animals.

In addition, the experimental predation studies and the release-recapture experiments share the common feature that mismatched animals are removed, or disappear more rapidly, from the gray shale than from the red sandstone substrate. This observation is difficult to interpret in the absence of detailed measurements of spectral reflectance of the substrates and animals. However, it may be that gray animals on the red substrate, while sharply contrasted in color, approximate in appearance spots of shadow produced by small objects, or perhaps dead twigs, and are thus not as easily recognized by predators.

These observations lead us to conclude that predator selection against mismatched animals is the major mechanism for maintaining substrate color matching in this species.

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